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Tissue-Specific ICAM-1 Expression and Neutrophil Transmigration in the Copper-Deficient Rat

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Abstract—Dietary copper deficiency promotes neutrophil accumulation in rat lungs. We have now investigated the potential mechanisms of this effect. Male weanling rats were fed a Cu-adequate (6.0 mg diet) or Cu-deficient diet (0.30 mg) for 4 wks. Endothelial intercellular adhesion molecule-1 (ICAM-1) expression was measured *in vivo* and *in vitro* using a radiolabeled monoclonal antibody to rat ICAM-1. Tissue neutrophil accumulation was measured by myeloperoxidase (MPO) content and neutrophil transendothelial migration was assessed *in vitro*. Dietary copper deficiency had no effects on the expression of ICAM-1 in lung, liver, heart, kidney, or cremaster. However, MPO content was significantly greater in the lungs of copper-deficient rats. Endotoxin-induced ICAM-1 expression was greater in the lungs and hearts of copper-deficient rats. Similarly, cultured rat endothelial cells that were Cu-chelated expressed more ICAM-1 after endotoxin. This correlated with the significant increase in MPO in lungs of copper-deficient rats treated with endotoxin. The results suggest a tissue-specific difference in ICAM-1 expression and neutrophil accumulation during inflammation in copper-deficient rats. The findings suggest that lung inflammatory mechanisms are particularly sensitive to copper deficiency.

KEY WORDS: Copper, Endothelium, ICAM-1, Inflammation, Neutrophils, lung

INTRODUCTION

Inflammation is a complex of reactions that involve blood vessels and adjacent tissue in response to injurious or infectious stimuli. Early in the inflammatory process blood vessels dilate, permeability to plasma proteins increases and leukocytes infiltrate the perivascular tissues. Each of these inflammatory processes is exaggerated by dietary copper restriction in experimental animals. Several investigators have reported an increase in carageenen-induced edema formation in copper-deficient rats (1). In our dietary copper-deficient rat model, we have observed an increase in mast cell-mediated protein extravasation from post-capillary venules (2, 3) and greater albumin leakage in the IgG immune complex lung injury model (4). We have also reported a significant increase in the dilation of arterioles and consequent hypotension during lipopolysaccharide (LPS)-induced endotoxemia in copper-deficient rats (5). In addition to the enhanced permeability and vasodilation during copper-restriction, neutrophil accumulation is significantly greater in the lungs of copper-deficient mice stimulated with LPS (6).

We have recently reported a significant increase in

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myeloperoxidase (MPO) activity in the lungs of unstimulated copper-deficient rats (4). These results indicate that neutrophil accumulation is increased as a result of the copper-deficient diet. However, we have also shown in the *in vivo* cremaster microcirculation, that the adhesion process between leukocytes and the vascular endothelium is depressed in response to the chemoattractant N-formyl-methionyl-leucyl-phenylalanine (fMLP) and to tumor necrosis factor-α (TNFα) (7). Therefore, in the current study we examined the role of dietary copper on constituitive and induced endothelial expression of the intercellular adhesion molecule-1 (ICAM-1) and on neutrophil extravasation. The study was conducted in five different organs to determine if there is a tissue-specific sensitivity to the effects of copper deficiency on the neutrophil-endothelial component of inflammation. Complimentary *in vitro* studies were also done examining ICAM-1 expression in control and Cu-chelated cultured endothelial cells and neutrophil extravasation through monolayers of cultured endothelial cells.

MATERIALS AND METHODS

Animals and Diet

This project was approved by the University of Louisville Animal Care and Use Committee and the male weanling Sprague-Dawley rats were purchased from Charles River Breeding Laboratories, Wilmington, MA. On arrival, rats were housed individually in stainless steel cages in a temperature- and humidity-controlled room with a 12-h light-dark cycle. The rats were given free access to distilled water and to one of two purified diets for 4 wk. The basal diet was a casein-sucrose-cornstarch-based diet (no. TD 84469, Teklad Test Diets, Madison, WI) containing all known essential vitamins and minerals except for copper and iron. The copper-adequate diet consisted of the basal diet (940 g/kg of total diet) with safflower oil (50 g/kg) and a copper-iron mineral mix that provided 0.22 g of ferric citrate (16% Fe) and 24 mg of $CuSO_4 \cdot H_2O$ per kilogram of diet. The copper-deficient diet was the same except for replacement of copper with cornstarch in the mineral mix. Diet analysis by inductively coupled argon plasma emission spectrometry (Liberty II model, Varian Associates, Sugarland, TX) indicated that the copper-adequate diet contained 6.18 mg copper/kg diet and the copper-deficient diet contained 0.29 mg copper/kg diet. Parallel assays of National Institute of Standards and Technology (NIST; Gaithersburg, MD) reference samples (citrus leaves, no. 1572) yielded values within the specified range, which validated our copper assays.

In Vivo Protocols

ICAM-1 Labeling

Forty rats (20 CuA and 20 CuD) were anesthetized with sodium pentobarbital (50 mg/kg i.p.) before surgery. The rats were separated into two groups, one group receiving 1 μg of radio-labeled mouse antibody to rat ICAM-1(^{125}I-ICAM-1 mAb (1A29)) and 4 μg of unlabeled ICAM-1 mAb. The other group received 1μg of the isotype-matched negative control (^{125}I-MOPC-31 mAb) and 4μg of unlabeled MOPC-31 mAb for determination of non-specific binding. Half of the animals in each group received an injection of endotoxin from *Salmonella abortus equi* with an incubation period of 5 hr before antibody injection. The antibodies were injected in the penile vein and allowed to circulate for 5 min. The animal was then perfused with 50 ml of saline via the superior vena cava while exsanguinated by transection of the inferior vena cava. The heart, liver, kidney, lung and cremaster muscle were then harvested and weighed. Activity for the radiolabeled MAb's was measured using a gamma counter (Packard Cobra II). The accumulated activity of each MAb in an organ was expressed as the percentage of the injected activity per gram of tissue (8).

Myeloperoxidase Assay

Whole organ myeloperoxidase (MPO) activity was quantitated as previously described (4). Briefly, 100 mg of tissue were homogenized and diluted in 50 mM potassium phosphate buffer containing 0.5% hexadecyltrimetheylammonium bromide, pH 6.0. After sonication and two freeze-thaw cycles, samples were centrifuged at 4000$\times g$ for 30 min. The supernatants were reacted with H_2O_2 (0.3mM) in the presence of tetramethylbenzidine (1.6 mM). MPO activity was assessed by measuring the change in absorbance at 655 nm with human MPO used as a standard.

Hepatic Copper Determination

After the collection of tissues, the median lobe of the liver was removed, weighed and frozen at -10°C for subsequent copper analysis. Tissues were lyophilized and digested in nitric acid and hydrogen peroxide (9). Hepatic copper concentrations of individual rats were

assessed by using inductively coupled argon plasma emission spectrometry (model 35608, Thermo ARL-VG Elemental, Franklin, MA). Parallel assays of reference samples (no. 1477a, bovine liver) from the NIST yielded mineral contents within the specified range.

In vitro protocols

Cell Culture System

Endothelial cells from rat coronary microcirculation were a courtesy gift from Cynthia Meininger, Ph. D. (Texas A & M University). Costar brand Transwell plate inserts with polycarbonate membranes (6.5 mm in diameter, 5 μm pore size and pore density of $2 \times 10^6/cm^2$; Corning Costar Corp. Cambridge, MA) were coated with fibronectin (Sigma, St. Louise, MO) for 1 hr. After the fibronectin treatment the Transwells were seeded with rat endothelial cells. The cells were grown in a complete medium consisting of Dulbecco's modified Eagle's medium (DMEM), 20% bovine serum, 4.5% glucose, 1% L-glutamine (final concentration 2mM) and sodium pyruvate (final concentration 2mM), 2 ml heparin (20 units/ml), and 1% antibiotics (penicillin and streptomycin; final concentration of 100 units each) until they formed a complete monolayer on the membrane surface. In order to reduce copper content in cultured endothelial cells, 50 μmol/L tetraethylenepentamine (TEPA) was added to the medium for 72 hr (10, 11). No cell growth factors were added to the culture. Cells were incubated at 37°C at 5% CO_2 in a humidified environment. This chelation method produces a 65% decrease in endothelial cell copper concentration (11).

Cell Adhesion Molecule Expression

Fluorescein isothiocyanate (FITC)-conjugated mouse anti-rat antibodies against ICAM-1 (CD54) were purchased from Pharmingen (San Diego, CA). Thirty μl of 2% fetal bovine serum (FBS) was added to 12 × 75 mm capped conical tubes. Four μl of FITC conjugated mouse anti-rat ICAM-1 antibody (0.5 mg/ml) was added to each tube. Tubes for isotype controls (mouse anti-rat IgG_1, κ) were prepared simultaneously. Endothelial cells were used as unstimulated controls or were pretreated with endotoxin (2 μg/ml for 5 hr). Fifty μl of washed endothelial cell suspension (1.0×10^6 cells) was added to tube and mixed gently. Cells were incubated on ice for 45 min, washed once in cold 2% FBS, and resuspended with 450 μl cold 2% paraformaldehyde solution. All steps were performed at 4°C. Fixed cells were stored at 4°C until FACS analysis within 3 days. FITC fluorescence was read by a FACScan flow cytometer with argon laser (Becton Dickinson, San Jose, CA). Data was collected for 10,000 cells using LYSIS II software (version 2.0 Becton Dickinson, San Jose, CA).

Data analysis was performed using WinMDI Flow Cytometry Application (Build #1301-19-2000, Version 2.8). Histograms were used to set the boundaries of control samples labeled with FITC isotype antibodies. The number of events in the marked region of specifically bound antibody was expressed as a percent of the total number of cells analyzed (10,000).

PMN Transmigration Assay

Animals (total n = 5 CuA and n = 5 CuD) were anesthetized with sodium pentobarbital (50 mg/kg of body weight; i.p.). The blood was withdrawn by venipuncture of the *vena cava* using syringes containing sodium citrate anticoagulant (final concentration of 10.9 mmol/L) with a ratio of 1 part anticoagulant to 9 parts of blood. A total of 5.5 ml freshly collected blood and the anticoagulant mixture was carefully layered on the top of the same volume (5.5 ml) of Polymorphprep (Nycomed Pharma, Oslo, Norway) in a 15 ml centrifuge tube. The sample was centrifuged at 550×*g* for 35 min at room temperature (~21°C). After the centrifugation PMNs were carefully collected from the lower band at the sample/medium interface using a Pasteur pipette. To restore normal osmolarity, the PMN fraction was diluted by addition of one volume of phosphate buffered saline (PBS) at twice of its normal concentration (42.6 mmol/L Na_2HPO_4, 7.4 mmol/L NaH_2PO_4, 90 mmol/L NaCl, 5 mmo/L KCL, pH = 7.4; 285 mosmol) containing 0.5% bovine serum albumin (BSA). The cells were washed 3 times by centrifugation at 500×*g* for 5 min each time in PBS (normal concentration). To minimize contamination of the PMNs with RBCs, after the last wash, pelleted cells were suspended in 1.0 ml of deionized water for 30 sec, and then to restore the osmolarity, *NaCl* solution at 2 normal concentration was added. The sample was centrifuged again at 500×*g* for 5 min and cells were separated from the supernatant. The cell pellet was resuspended in 1 ml of PBS and 5 μl of 2',7'-bis (2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF, AM; Molecular Probes, Eugene, OR) was added. The cell suspension was incubated at 37°C for 30 min with slow agitation. After the incubation, to remove excess fluorescence, PMNs were

centrifuged at 500×*g* for 5 min, the supernatant was discarded and the cells were suspended in 1 ml of PBS containing 0.5% BSA. The PMNs labeled with the fluorescence were counted in a hemocytometer. The hemocytometer was placed on the stage of a Carl Zeiss Axioscope FS upright fluorescent microscope (Carl Zeiss, Germany) equipped with 40× water immersible, UV transmissible fluorescent objective (n.a. 0.75). Fluorescence on the cell surface was visualized using epi-illumination with blue light (excitation at 495 nm wavelength) from a mercury arc lamp and observation of the fluorescence at 520 nm emission wavelength. We did not find contamination of our samples with fluorescently labeled erythrocytes. Only the fluorescently labeled PMNs were counted. For the experimentation, the number of cells was adjusted to 10^6 cells per ml of PBS-0.5% BSA solution.

Prior to experimentation, endothelial cells were washed with Hank's balanced salt solution (HBSS) containing 10 mM HEPES (pH 7.4) or TEPA respectively. Then a PBS-BSA mixture was used for the final washing. For the PMN transmigration assay, we used the method of Arthington et al. (12). 200 μl of PBS-BSA-PMN suspension was placed in the Transwells and incubated for 1 hr at 37°C. PBS-BSA containing *f*MLP (10 nM) chemoattractant peptide was stirred constantly (at 400 rpm) with a magnet stirring bar in the lower chamber of the Transwell. All the experiments were done in duplicate.In our preliminary studies we did not find neutrophil migration through the endothelial-membrane layer in the absence of chemoattractant in the lower chamber. After the incubation a solution from the lower chamber was collected and the number of PMNs were counted. The results were averaged for each animal/experiment. The difference between the initial number of PMNs in the upper chamber and the number of cells found in the lower chamber after the transmigration were calculated and expressed as a per cent of the total number of PMNs before the experiment.

Statistics

All data are expressed as mean ±SE. Comparisons between dietary groups were by Student's t-test. Two-way analysis of variance (ANOVA) was used to assess effects of dietary copper and LPS treatment. If ANOVA showed interactions, Tukey's test was used for pairwise comparisons (SigmaStat Statistical Software, SPSS, Chicago, IL). Differences were considered significant when $p < 0.05$.

Table 1. Body Weight and Copper Status Indices of Rats Fed the Copper-Adequate and Copper-Deficient Diets for 4 Weeks.

Variable	Copper-adequate	Copper-Deficient
	n = 5	n = 5
Body wt, (g)	230 ± 6	208 ± 5
Liver copper, (mg/g dry wt)	12.06 ± 0.38	1.98 ± 0.16*
Heart wt/body wt, (mg/g)	3.72 ± 0.06	7.20 ± 0.41*

Values are mean ± SEM. *p < 0.05 compared to the copper-adequate group.

RESULTS

Establishment of Copper Deficiency

Rats fed the copper-deficient diet for 4 weeks developed anemia and had significantly lower liver copper concentration and a higher heart wt/body wt compared with the copper-adequate fed control group (Table 1). These markers are indicative of copper-deficiency.

Effects of Copper-Deficiency on Intrinsic and Stimulated Endothelial ICAM-1 Expression

In order to determine if copper-deficiency itself causes any alteration in the expression of ICAM-1 in different vascular beds, specific binding of ^{125}I-labeled anti-ICAM-1 was measured. Each tissue exhibited a different amount of anti-ICAM-1 binding with the kidneys having the greatest amount of binding and the heart the least amount of binding (Table 2). These results suggest heterogeneous expression of ICAM-1 in the rat vasculature. However, there were no significant differences in ICAM-1 expression in similar organs from copper-adequate and copper-deficient rats treated with the control vehicle. In rats fed a copper-adequate diet, challenge with endotoxin had no effect on ICAM-1 expression in any organ (Table 2). In contrast, in rats fed a copper-deficient diet, endotoxin treatment resulted in significant increases in ICAM-1 expression in lung and heart, but not liver, kidney or cremaster muscle.

When rat endothelial cells were cultured under normal conditions, treatment with endotoxin had no effect on ICAM-1 expression (Fig. 1). Endothelial cells cultured in the presence of TEPA, which causes a 65% reduction in cell copper concentration, had a similar amount of ICAM-1 expressed as their copper-adequate counterparts. This suggests that copper-deficiency does

Table 2. ICAM-1 Expression under Control and Endotoxin-Stimulated Conditions in Rats Fed the Copper-Adequate (CuA) and Copper-Deficient (CuD) Diets for 4 Weeks.

Treatment (n)	Lung	Liver	Kidney	Heart	Cremaster
CuA (5)	1.43 ± 0.09^{A}	0.90 ± 0.03	3.11 ± 0.30	0.48 ± 0.02^{A}	0.76 ± 0.07
CuD (5)	1.41 ± 0.09^{A}	0.90 ± 0.05	3.58 ± 0.26	0.46 ± 0.02^{A}	0.68 ± 0.01
CuA+LPS (5)	1.24 ± 0.05^{A}	0.87 ± 0.09	3.09 ± 0.11	0.44 ± 0.03^{A}	0.82 ± 0.04
CuD+LPS (5)	1.76 ± 0.08^{B}	0.94 ± 0.06	2.72 ± 0.36	0.57 ± 0.04^{B}	0.74 ± 0.04

Values are mean ± SE percent injected dose/g tissue. ANOVA showed interactions between Cu and LPS on lung and heart ICAM-1. Values with different letters are significantly different ($p < 0.05$, Tukey test), compared to other values in the same column.

not, by itself, result in increased ICAM-1 expression. However, in response to endotoxin treatment, ICAM-1 expression on copper-deficient endothelial cells was 2-fold greater than unstimulated cells and their copper-adequate counterparts (Fig. 1). These data suggest that copper-deficiency may prime the signal transduction pathways utilized by endotoxin.

Enhanced Neutrophil Transmigration and Pulmonary Accumulation Induced by Copper-Deficiency

In order to determine if the differential expression of ICAM-1 on the pulmonary and coronary vasculatures observed in Table 2 was associated with increased neutrophil accumulation in those tissues, we measured tissue levels of MPO, a specific index of neutrophil accumulation. In animals treated with the control vehicle, MPO activity was 4-fold greater in the lungs and 3-fold greater in the kidneys of the copper-deficient rats compared to the copper-adequate group (Table 3). None of the other organs showed an effect of copper deficiency on MPO content in the absence of endotoxin treatment. Treatment of copper-adequate rats with endotoxin resulted in significant increases in the MPO content of all tissues except the kidneys compared to those of copper-adequate rats treated with the control vehicle (Table 3). It is notable that only in the lung were main effects of both LPS and copper deficiency apparent, indicating that only in this tissue is MPO content, and hence neutrophil invasion additive for the two treatments.

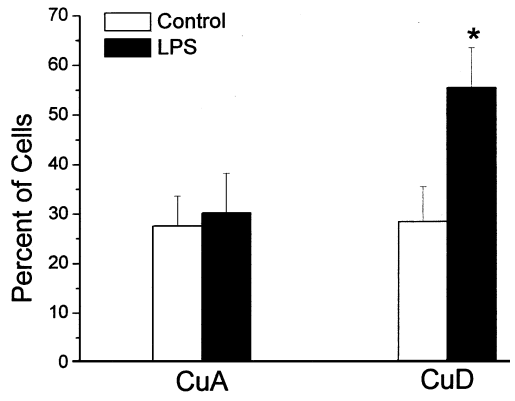

Fig. 1. The percent of cultured endothelial cells expressing ICAM-1 adhesion molecule with and without LPS stimulation. CuA; the copper-adequate control endothelial cells. CuD; the copper-deficient cells which were chelated with 50 mol TEPA for 72 hr prior to experimentation as described in Materials and Methods. ANOVA showed an interaction between Cu and LPS on ICAM-1 expression. *$p < 0.05$ (Tukey test) for comparison of copper-deficient group to the copper-adequate control group.

We next assessed whether copper-deficiency may affect neutrophil function and the capacity to transmigrate across the endothelium. The ability of neutrophils from copper-adequate and copper-deficient rats to transmigrate across monolayers of normal rat endothelial cells was assessed using fMLP as the chemoattractant. Approximately 7.5% of all copper-adequate neutrophils transmigrated across endothelial monolayers in response to fMLP (Fig. 2). In contrast, nearly 12% of all copper-deficient neutrophils transmigrated across endothelial monolayers ($p < 0.05$) (Fig. 2). These data suggest that the effects of copper-deficiency on neutrophils promotes transendothelial migration.

DISCUSSION

The microvascular response to inflammatory stimuli includes the adhesion and transmigration of leukocytes from the blood to sites of inflammation. This adhesion process involves a series of events that occur along the vessel wall. Firm adhesion or sticking of leukocytes

Table 3. Organ Myeloperoxidase Activity in Rats Fed a Copper-Adequate (CuA) or Copper-Deficient (CuD) Diet for 4 Weeks and Then Treated with Vehicle Control or LPS.

Treatment	Lung	Liver	Kidney	Heart	Cremaster
CuA (5)	0.39 ± 0.11^{A}	0.027 ± 0.006^{A}	0.032 ± 0.003^{A}	0.05 ± 0.002^{A}	0.031 ± 0.006^{A}
CuD (5)	1.68 ± 0.30^{B}	0.023 ± 0.001^{A}	0.100 ± 0.037^{B}	0.04 ± 0.003^{A}	0.035 ± 0.003^{A}
CuA + LPS (5)	1.82 ± 0.13^{B}	1.189 ± 0.213^{B}	0.053 ± 0.004^{A}	0.075 ± 0.016^{B}	1.66 ± 0.413^{B}
CuD + LPS (5	2.36 ± 0.06^{C}	0.681 ± 0.136^{B}	0.127 ± 0.035^{B}	0.081 ± 0.010^{B}	1.85 ± 0.303^{B}

Values are mean ± SE U/g tissue. ANOVA showed significant main effects of Cu on lung and kidney MPO, significant main effects of LPS on lung, liver, heart and cremaster MPO. Values with different letters are significantly different ($p < 0.05$, Tukey test) compared to other values in the same column.

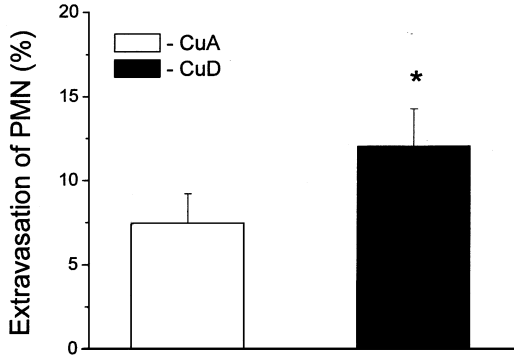

Fig. 2. Extravasation of polymorphonuclear leukocytes (PMNs) from copper-adequate ($n = 5$) and copper-deficient ($n = 5$) rats through a monolayer of cultured endothelial cells. Values are mean SEM. $*p < 0.05$ for comparison of copper-deficient group to the copper-adequate control group.

to the endothelium prior to transmigration is primarily mediated by the engagement of leukocyte integrins to members of the immunoglobulin superfamily on the endothelial cell surface. Intercellular adhesion molecule-1 (ICAM-1) is a counter receptor for the CD11b/CD18 integrins on neutrophils and is constitutively expressed on resting endothelium (13). The expression of ICAM-1 is enhanced by inflammatory cytokines including those released during exposure to LPS.

In the current study, the constitutive expression of ICAM-1 was not different between the copper-adequate and copper-deficient rats in the tissues examined (Table 2). Similar results were seen with cultured microvascular endothelial cells that were either normal or Cu-chelated to mimic the copper-adequate and copper-deficient rat models (Fig. 1). While constitutive expression of ICAM-1 was not altered by the dietary copper deficiency, there was significantly more neutrophil accumulation in the lungs and kidneys of the copper-deficient group as indicated by MPO activity (Table 3). These results suggest a neutrophil-endothelial cell adhesion that is independent of the CD11b/CD18-ICAM-1 interaction in the lung and kidney.

Unlike the systemic circulation, neutrophil migration through post-capillary venules in the pulmonary and renal circulation is not solely dependent on the expression of the neutrophil CD11b/CD18 complex (14, 15). A CD11b/CD18-independent adhesion pathway that is not stimulated by LPS also exists in both the lung and kidney circulations (15–17). Activation of this non-endotoxin stimulated adhesion pathway by dietary copper-restriction may be the mechanism by which neutrophils preferentially accumulated in the lungs and kidneys of the nonstimulated copper-deficient group. The augmented neutrophil-endothelial interactions in the lung may contribute to pulmonary pathologies involving neutrophils such as the greater susceptibility of copper-deficient neonatal rats to develop acute respiratory distress syndrome (ARDS) (18).

When ICAM-1expression was induced with endotoxin, there was significantly greater binding of the anti-ICAM-1 antibody in the lungs and hearts of the copper-deficient group compared to the copper-adequate controls (Table 2). These *in vivo* results demonstrate a tissue-specific heterogeneity in the expression of endothelial cell ICAM-1 to inflammatory stimuli similar to that reported elsewhere (8). The *in vitro* cell culture model also showed that endotoxin induced ICAM-1 expression on Cu-chelated microvascular endothelial cells was significantly greater than on the normal endothelial cells. Coincident with the enhanced endotoxin-induced ICAM-1 expression in the copper-deficient group, neutrophil accumulation in the lungs of copper-deficient rats was significantly greater than in the copper-adequate controls (Table 3). Combined, the *in vivo* and *in vitro* results suggest an alteration of the copper-deficient endothelium that promotes neutrophil-

endothelial cell adhesion in the lung and heart vasculature during inflammation. Further, the results suggest that dietary copper-restriction may cause a priming of the endothelial cell signal transduction pathway utilized by endotoxin to induce ICAM-1 expression.

While the greater ICAM-1 expression indicates an alteration of the copper-deficient endothelial cell, changes in the copper-deficient neutrophil may also contribute to the enhanced neutrophil-endothelial interactions. In the current study, copper-deficient neutrophils migrated more readily through a monolayer of normal endothelial cells than did the copper-adequate neutrophils (Fig. 2). Similar increased neutrophil chemotaxis has been reported *in vivo* in the copper-deficient heifer (12). The concept that the sequestration of neutrophils is caused by the effects of copper-deficiency on both endothelial cells and neutrophils is further supported by the tissue-specific increases in MPO activity in the copper-deficient group (Table 3).

Our current data demonstrating augmented ICAM-1 expression and greater neutrophil transmigration extend our understanding of the enhanced inflammatory response in copper-deficient rats (2–4). The data suggest that copper-deficiency primes the lung inflammatory response by increasing the number of neutrophils in the non-inflamed lung. The combination of augmented neutrophil chemotaxis and endotoxin-induced ICAM-1 expression contributes to increased adhesion of neutrophils in the copper-deficient lung. Thus, it appears that the lung is particularly sensitive to inflammatory perturbations during conditions of copper deficiency.

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